

Investigation on biological action of Small-flowered Willowherb

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Small-flowered Willowherb (*Epilobium parviflorum* Schreb.) is traditionally used in the prevention and complementary treatment of benign prostatic hyperplasia (BPH). BPH is maintained by complex and still not entirely discovered pathological factors, but age-related hormone imbalance, decreased apoptosis, inflammation and excessive oxidative stress are definitely involved in the pathomechanism. Based on our analysis, willowherb is rich in various structured phenoloids. Its most characteristic compounds are myricetin-, quercetin- and kaempferol-glycosides, as well as the often macrocyclic derivatives of ellagic- and gallic acid (e.g.: oenothlein B). Apolar extract of willowherb contains β -sitosterol.

Our workgroup aimed to investigate the mechanism of action of *Epilobium parviflorum* with special regard to its antioxidant activity and anti-inflammatory effect.

H-donor capacity of willowherb was measured by two different spectrophotometric methods (ABTS, DPPH). Inhibitory action on lipidperoxidation was examined with TBA assay, on bovine brain liposomes. Antioxidant cell-protective effect of *Epilobium* was studied on fibroblast cells. Anti-inflammatory effect was investigated on macrophage cells, by examination of COX-enzyme inhibitory action of extract.

Willowherb showed a remarkable H-donor activity (EC_{50} : ABTS $1.71 \pm 0.05 \mu\text{g/ml}$; DPPH $3.01 \pm 0.03 \mu\text{g/ml}$), comparable to that of ascorbic acid and trolox. In the TBA assay willowherb extract showed concentration-dependent inhibition of lipid peroxidation at doses over 0.20 mg/mL ($IC_{50} = 2.37 \pm 0.12 \text{ mg/mL}$). *Epilobium* extract exerted steady and concentration-dependent protective effect against oxidative damage generated on fibroblast cells. The protective action was comparable to that of catalase enzyme (250 IU/ml). *Epilobium* extract showed concentration-dependent COX-enzyme inhibitory action ($IC_{50} = 1.4 \pm 0.1 \mu\text{g/ml}$).

Biological action of *Epilobium parviflorum* has been *in vitro* investigated. Based on our results, willowherb possessed high H-donor capacity and antioxidant cell-protective effect. The extract inhibited lipidperoxidation and the activity of COX-enzyme. These results suggest that extract of *Epilobium parviflorum* has antioxidant and anti-inflammatory properties which are likely to contribute to its beneficial effect in BPH. However, for wider, evidence-based application of willowherb further *in vitro* and *in vivo* studies are necessary.

Pigment photosensitized reactions make dark-grown pea epicotyls wilt in the light – direct detection of ROS promoting type-I and type-II photochemistry

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Upon illumination, epicotyls of dark grown pea (*Pisum sativum* L.) seedlings loose turgor in their middle section and wilt. Direct detection of singlet oxygen shows the involvement of type-II photoreactions, while co-localization of hydrogen peroxide and protochlorophyllide monomers suggests the contribution of type-I photodynamic pigment reactions as well. Hydroxyl radicals were detectable with spin trapping electron paramagnetic resonance spectroscopy and were also triggered by adding hydrogen peroxide in the dark, demonstrating Fenton chemistry.

In plants, native arrangements of pigment-protein complexes are critical during early plant development. In most angiosperms, various chlorophyllous pigments are safely stored in aggregates (macrodomains), such as prolamellar bodies of leaf etioplasts, to prevent photo-oxidation. However, etioplasts in epicotyls or other stem related organs contain only few and small macrodomains and the chlorophyll precursor pigments, such as protochlorophyllide (Pchl) are predominantly in monomer state in them.

Seven days old pea (*Pisum sativum* L.) epicotyls were germinated and grown in darkness. Singlet oxygen ($^1\text{O}_2$) production was visualized with DanePy (3-[N-(β -diethylaminoethyl)-N-dansyl] aminomethyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrole) fluorescence. H_2O_2 was detected by DAB (3,3'-diaminobenzidine) staining or by transition electron microscopy (TEM) using CeCl_3 . Hydroxyl radicals ($\cdot\text{OH}$) were detected with spin trapping EPR spectroscopy using POBN or on the basis of HTPA (hydroxyl-terephthalate) fluorescence. Protochlorophyllide (Pchl) localization was detected by fluorescence microscopy, its monomers/oligomers were identified by low temperature fluorescence emission spectra.

Illumination caused fast turgor loss and wilting in middle segments of the epicotyls accompanied with accumulation of water in the intercellular cavities. During this process, porphyrin-type pigments were gradually bleached, while $^1\text{O}_2$ and lipid peroxidation products were detected suggesting a type-II, porphyrin (Pchl or Chl) -photosensitized mechanism.

On the other hand, selective assays showed the presence of three different ROS: $\text{O}_2^{\cdot-}$, H_2O_2 and $\cdot\text{OH}$ in the illuminated pea epicotyls, preferentially in the mid-sections. H_2O_2 was mainly produced along the radial walls of cells in areas also rich in monomer Pchl. Although $\cdot\text{OH}$ production, which was restricted to the mid-section was light-dependent, the $\text{H}_2\text{O}_2 \rightarrow \cdot\text{OH}$ conversion also occurred without illumination, showing the presence of Fenton-catalysts in this region. These data demonstrate that products of a type-I photoreaction also contribute to disordered water status of the epicotyls and their wilting in light.

TIP47 protects of mitochondrial membrane integrity and inhibits oxidative stress-induced cell death

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The intracellular role of tail-interacting protein of 47kDa (TIP47/PP17b) has been controversial, no data are available for its possible role in tumor development, or in regulation of cell death. TIP47 is expressed in almost if not all tissues. During pregnancy, TIP47 serum levels increase; after birth they drop. Although some TIP47 is found on lipid droplets, it is known to be required for the delivery of mannose 6-phosphate receptors from late endosomes to the Golgi, both *in vitro* and in living cells. The protein binds the cytoplasmic domains of the cation-dependent and cation-independent receptors, and is recruited to late endosomes by binding to Rab9 GTPase. The loss of TIP47 destabilizes Rab9 which is also required for proper receptor transport.

The aim of this study was to find an intracellular role of this protein.

The vector containing TIP47, truncated-TIP47 or the empty pcDNA3.1 vector was transfected into NIH3T3 cells. Cells were treated with H_2O_2 and cell viabilities were measured by MTT-viability assay. TIP47 was silenced by dicer-siRNA in HeLa cells. Mitochondrial membrane potential was monitored on isolated rat liver mitochondria *in vitro* by fluorescence of Rh123 or on TIP47 transfected and treated NIH3T3 cells *in vivo*. Depolarization of mitochondria can be visualized *in vivo* by using the membrane potential sensitive dye, JC-1 by fluorescent microscopy. Ratio of apoptosis and necrosis were evaluated after double staining with fluorescein isothiocyanate (FITC)-labeled annexin V and propidium iodide using flow cytometry and fluorescent microscopy.

TIP47 was over-expressed in a cell line normally not expressing it (NIH3T3), or suppressed by small interfering RNA in a cell line that normally express TIP47 (HeLa) at high extent before exposing cells to oxidative stress. Over-expression of TIP47 prevented hydrogen-peroxide induced cell death and the collapse of mitochondrial membrane potential. Suppression of TIP47 synthesis by small interfering RNA technique sensitized the HeLa cells to hydrogen peroxide induced cell death. We proved with both *in vitro* and *in vivo* experiments that TIP47 caused hyperpolarisation of mitochondrial membrane and reduced Ca^{++} induced mitochondrial membrane depolarization. In view of the fact that mitochondria may role in both apoptotic and necrotic cell death, we used flow cytometry to determine the percentage of apoptotic and necrotic cells. The results unambiguously justified protective effects of TIP47 protein.

We provided evidence that TIP47/PP17b can bind to mitochondria and can protect mitochondrial membrane integrity, as well as can prevent oxidative stress induced cell death providing the first evidence the possible oncogenic property of TIP47/PP17b.